Whole Mouse Skin

RNA ISOLATION USING TRIZOL REAGENT FOR TOTAL RNA and QIAGEN Mini-Column Clean-Up

Skin Harvest:

Work as quickly as possible until tissue is frozen.

Sacrifice mouse; immediately shave. Cut out skin from the back (~4cm x 4cm, or 1 1/2" x 1 1/2"). Remove a small piece for histology. Quickly place remaining section on a clean weigh boat and weigh. Transfer immediately to the waiting liquid nitrogen in the mortar, and proceed with the following steps.

Total RNA Extraction:

Volumes are based on skin sizes and weights mentioned above.

1. Place whole mouse skin into a mortar, filled with liquid nitrogen, that is sitting in a bucket of dry ice.
2. Grind the skin with a cold pestle, allowing the liquid nitrogen to sublimate without adding more. Keep grinding until the skin is a powder-form.
3. Scrape powder into a pre-chilled tube (on dry ice) and keep in dry ice, or store at -70^{o}C, until you are ready to extract with TRIzol (Gibco BRL).

Tissue Homogenization

Keep homogenization tubes containing measured TRIzol on wet ice. Clean homogenizer before use, between samples and after use with 0.1M NaOH then 1M Tris, then autoclaved, Rnase-free water. Homogenize well with tube submerged in a beaker of wet ice.

1. Add 1 ml of TRIzol Reagent per 50-100 mg of initial tissue weight to homogenzation tubes that are kept cold by sitting in wet ice.
2. Transfer frozen, powder-form skin to waiting TRIzol Reagent; vortex vigorously
3. Homogenize thoroughly (until it is one homogeneous solution). Foaming of the sample should be avoided. Proceed to next step.

Phase Separation

1. Incubate homogenized samples for 5 minutes, RT.
2. Add 0.3 ml chloroform (Molecular Biology grade, Sigma C2432) per 1 ml of TRIzol Reagent originally used.
3. Mix vigorously by hand for about 30 seconds; incubate, RT, for 3 minutes.
4. Centrfuge samples at 12,000 x g (~10,000 rpm),15 minutes, 4^{o}C.
5. Pipette off the top aqueous phase (RNA) – avoid protein interface, it will be big - and transfer to a new 13 ml homogenization / centrifuge tube. This will be ~60% of the volume of TRIzol used.
6. Add 1:1 (extracted aqueous phase to isopropyl alcohol. Cap and invert 10x to mix.
7. Incubate for 10 minutes, RT.
8. Centrifuge samples at 12,000 x g (~10,000 rpm), 10 minutes, 4^{o}C.
9. RNA precipitate forms a gel-like pellet on the side and bottom of the tube.
10. Pour off the supernatant.

11. Wash the RNA pellet once with 600 µl of 75% RNase-free prepared ethanol (Sigma E702-3). Centrifuge <7500 x g (~8500 rpm), 5 minutes, 4^{o}C.
12. Gently pour off ethanol and place tube upside down on a clean paper and allow to air-dry for 15 minutes.
13. Resuspend pellet in (50, 100, or 200 µl) RNase-free water depending on the size of the pellet. Heat 10 minutes, 60^{o}C, to facilitate resuspension.

Once dissolved, always keep RNA on ice and store at -70^{o}C in small aliquots to minimize freeze/thaw cycles.

Qiagen Clean-Up:

Refer to the Qiagen Mini-column handbook for details of this protocol.

Further purify RNA using this protocol. This protocol reduces DNA contamination and dilutes salt carry over. Mini columns (2-3 columns/sample: do not overload!) are preferred because they yield a greater recovery rate.

Quantitate and store at -70^{o}C.

Quantitation of total RNA

The concentration of RNA should be determined by measuring the absorbance at 260nm in a spectrophotometer. Expected yield from 4 x 4 cm size of skin (250–500 mg) is 250-500µg total RNA (~1µg/ml).

Purity of RNA

The ratio of the readings at 260nm and 280nm provides an estimate of the purity of RNA. A 260/280 ratio of 1.6 to 1.7 is good for RNA in water (it should be higher if in buffer).

Integrity of RNA

The integrity and size distribution of total RNA should be checked by denaturing agarose gel electrophoresis and ethidium bromide staining. Take a photograph of the gel to submit with your samples.

For Agilent Commercial Arrays:

Aliquots of 40-50µg should be stored at ~1 µg/µl in RNase-free water.